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Letter to the Editors

Variegation associated with *lacZ* in transgenic animals: a warning note

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The *Escherichia coli lacZ* gene, which encodes the β-D-galactosidase enzyme (EC 3.2.1.23), is one of the most widely used reporter genes in transgenic studies. Many spatial and temporal expression patterns of genes and their developmental profiles have been identified using *lacZ* reporter transgenes. In these constructs, a subset of regulatory sequences from a given gene is forced to drive the expression of *lacZ* to the cells where the gene of interest would be normally expressed. Subsequently, the presence of β-D-galactosidase activity can be revealed by means of a simple biochemical assay that turn cells blue where *lacZ* gene is expressed. These fusions of eukaryotic sequences with the bacterial *lacZ* gene have been instrumental in the analysis of the complex regulatory elements controlling the expression of vertebrate genes. As a result of these analyses, *lacZ* has become the most common reporter gene used in developmental studies, especially in transgenic mice. However, some limitations have been observed in postnatal studies with *lacZ* in transgenic animals. In adults, *lacZ* transgenes often function poorly and display variegated expression whereas during embryogenesis *lacZ* sequences are efficiently expressed (Cui et al., 1994). The latter includes gene-trap experiments and knock-in strategies, which, apparently, do not seem to be associated with variegation events. It is important to note that variegation can be monitored only when expression of a transgene is evaluated on a cellular basis. If the expression of a transgene is examined in extracts from entire tissues, differences in the proportion of cells expressing the transgene will be interpreted as differences in the overall expression level of that tissue.

Several studies have reported somatic mosaicism in the expression of *lacZ* in postnatal transgenic animals (for example: Ramirez et al., 1994; Montoliu et al., 1995, Sutherland et al., 1997). Figure 1A shows a typical image from a standard *lacZ* staining procedure applied to an histological section of an adult transgenic mouse liver from an established line (Montoliu et al., 1995). According to the regulatory sequences included in that transgene, obtained from the enhancers of the mouse α-fetoprotein gene and cAMP response elements, the expression of the reporter *lacZ* gene was expected to occur in hepatocytes, with a zonal gradient towards the central vein. In contrast, only a limited amount of these cells stained blue. Indeed, a variegation pattern was observed resulting in groups of positive (blue) and negative cells. These results could not be attributed to a given threshold level in the β-galactosidase activity since a comparable pattern of expression was seen by *in situ* hybridization techniques using antisense *lacZ* riboprobes. Similar observations have been made in transgenic mice expressing *lacZ* under the control of regulatory sequences from K5, a keratin gene specific of the basal layer of stratified epithelia (Ramirez et al., 1994). Figure 1B shows the heterocellular expression pattern of *lacZ* in the oesophagus of a K5-*lacZ* transgenic mouse, in contrast to the activity of the endogenous K5 in every cell of the basal layer forming a ring-like structure, as determined by antibody staining (Ramirez et al., 1994; Ramirez & Vidal, unpublished). These variegated expression patterns resemble, to some extent, those observed in female mice with transgenic constructs inserted in the X-chromosome (i.e. Tan et al., 1995).

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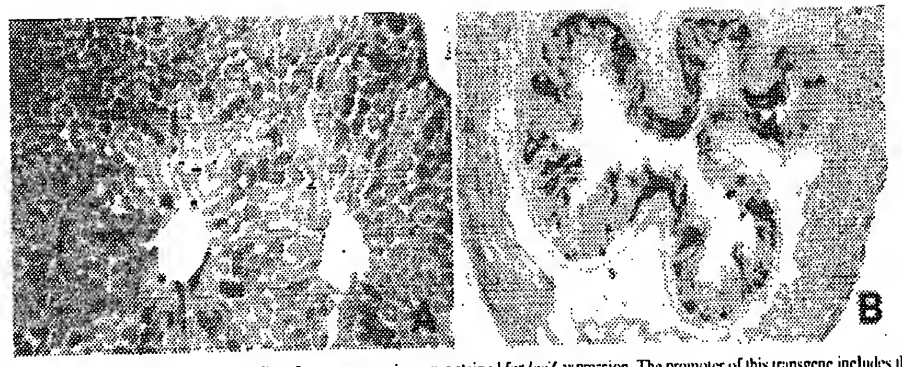

Figure 1. (A) Cryostat section of an adult liver from a transgenic mouse stained for *lacZ* expression. The promoter of this transgene includes the enhancer from the mouse α-fetoprotein gene and cAMP response elements driving the expression of *lacZ* (Montoliu et al., 1995; Photography by L. Montoliu & G. Schütz). (B) Cryostat section of an adult oesophagus from a transgenic mouse stained for *lacZ* expression. The promoter and regulatory regions of this transgene are derived from the bovine keratin 5 gene (Ramirez et al., 1994; Ramirez & Vidal, unpublished).

What could be the cause of the variegated expression observed with *lacZ* constructs in transgenic animals? Several explanations have been discussed in the literature. To begin with, the usual tandem-repeat nature of the transgene insertion appears to contribute to the variegation phenomenon by potentially triggering a stochastic silencing of transgenes, thereby decreasing the proportion of cells that express the transgene (Garrick et al., 1998).

Next, standard transgenic constructs are often prepared with limited amount of poorly characterized regulatory sequences, a procedure which favours the appearance of position effects from sequences surrounding the integration site, thus altering the expected expression pattern of the transgene. These position effects can be overcome by including complete expression domains in transgenes based on artificial chromosome-type of vectors (YACs, BACs or PACs), which would then guarantee the presence of all regulatory sequences that are relevant for the proper expression of the locus (i.e. Hiemisch et al., 1997). Moreover, the addition of specific regulatory sequences to standard *lacZ* transgenes increases the proportion of cells with β-galactosidase activity and decreases the extent of variegation (Sutherland et al., 1997). Taken together, these results would suggest that the observed variegation in *LacZ* constructs, like for other conventional transgenes, could be explained, at least in part, by the lack of regulatory elements needed to achieve optimal levels of expression. However, this is probably not the final answer since the few examples of YAC-based *lacZ* transgenes reported to date have been evaluated during embryo development and not examined in adult stages where potential variegation could appear.

Hence, it is not clear what makes *lacZ* gene especially prone to variegate in its expression. *lacZ* is a rather large (> 3 kb) prokaryotic gene with a high G + C content (close to 60%), a proportion higher than the value encountered in most mammalian genes. Methylation mediated silencing might be a clue to understand *lacZ* variegation (see Cohen-Tannoudji et al., in this issue), but experiments done in *Saccharomyces cerevisiae*, an eukaryote lacking DNA methylation, have revealed that *in vivo* transcription over the *lacZ* gene in eukaryotic cells do not progress very efficiently either. Transcript elongation of *lacZ* sequences in yeast is inhibited in a promoter-independent manner thus decreasing the expression of β-galactosidase. Moreover, *lacZ* transcription is very sensitive to yeast mutations affecting transcription elongation like *hpr1* (Chavez & Aguilera, 1997). Due to these transcriptional problems, *lacZ* becomes very unstable in *S. cerevisiae*, especially when located between repeats or in episomes. This instability is transcription-dependent and, although maximal in *hpr1* mutants, is clearly visible in wild-type strains (Chavez & Aguilera, 1997). In contrast with most genes present in the yeast nucleus, the exogenous *lacZ* does not show a chromatin structure with regularly positioned nucleosomes and its pattern of nuclease digestion is indistinguishable from naked DNA (Chavez, unpublished). Whether this unorganised chromatin structure or the high G + C content *per se* has something to do with the *lacZ* transcriptional problem is not clear, but it is worth mentioning that an eukaryotic

homologue of *lacZ* with lower G + C content behaves more stably and is transcription-prone in *S. cerevisiae* (Chavez & Aguilera, unpublished).

In conclusion, it appears that *lacZ* by itself may sum up to the natural tendency of conventional transgenes (with an incomplete set of regulatory sequences) to be expressed in a variegated fashion. Therefore, some prudence should be taken into account before using *lacZ* as reporter gene for transgenesis. Whereas *lacZ* could still be used confidently for most developmental studies its application to postnatal (and adult) experiments should be reconsidered. Alternative reporter systems more suitable for postnatal studies are the green-fluorescent protein, firefly luciferase and the alkaline phosphatase from human placenta. Further studies are required in order to understand the differential behaviour of *lacZ* sequences in transgenic animals during embryogenesis and in postnatal stages.

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